Frequency of \textit{mecA} Gene and Borderline Oxacillin Resistant \textit{Staphylococcus aureus} in Nosocomial Acquired Methicillin Resistance \textit{Staphylococcus aureus} Infections

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**Abstract:** The aim of the study was to determine the frequency and type of MRSA strains and antibiotic susceptibility in Al-Zahra Hospital, Isfahan, Iran. In an analytic descriptive survey in 2005 and early 2006, patients admitted to the hospital who contracted \textit{S. aureus} nosocomial infections were enrolled in the study. All isolates were identified by the conventional laboratory tests. Minimal Inhibitory Concentration (MIC) of oxacillin on isolated bacteria was determined by E-Test method. According to Clinical and Laboratory Standard Institute (CLSI) criteria all strains with MIC of \( \geq 4 \) \( \mu \text{g} \) for oxacillin were identified as MRSA. Intrinsic high level resistance (\textit{mecA} positive) and borderline oxacillin resistant \textit{Staphylococcus aureus} (BOSA) were detected by amoxicillin-clavulanate E-test strips. Strains with MIC of \( \geq 4 \) \( \mu \text{g} \) for oxacillin and \( \geq 8 \) \( \mu \text{g} \) for amoxicillin-clavulanate were identified as \textit{mecA} positive MRSA. Other staphylococci with MIC \( > 4 \) \( \mu \text{g} \) for oxacillin and \( \leq 4 \) for amoxicillin-clavulanate were identified as \textit{mecA} negative MRSA (BOSA). MIC of vancomycin also was determined on isolated bacteria. Data were analyzed by SPSS version 13 and WHO net version 5. Out of 134 \textit{Staphylococcus aureus} samples which were isolated from nosocomial infections 90 (67.2\%) were MRSA. Sixty seven out of 90 (74.5\%) MRSA were \textit{mecA} positive and 23 out of 90 (25.5\%) were \textit{mecA} negative (BOSA). Although most of the MRSA strains were isolated from surgical site infections, there were no statistically significant differences between types of \textit{Staphylococcus aureus} growing from variant sites of infections. Only one (1.49) of the \textit{mecA} positive MRSA had reduced susceptibility to vancomycin but all of the \textit{mecA}-negative MRSA (BOSA) were sensitive to it. Because one fourth of our staphylococci strains are \textit{mecA} negative BOSA and there is no alternative for vancomycin against \textit{mecA} positive MRSA and \textit{Enterococcus} spp. in our hospital, vancomycin should be reserved only for life threatening infections due to these organisms. Thus MRSA typing should be done to choose appropriate antibiotic for optimal treatment of MRSA infections.

**Key words:** Methicillin resistant \textit{Staphylococcus aureus}, nosocomial infections, \textit{mecA}, BOSA, vancomycin

**INTRODUCTION**

\textit{Staphylococcus aureus} plays key roles in causing various human infections. Severe nosocomial staphylococcal infections are encountered frequently and can result in significant morbidity and mortality (Kluimans et al., 1997). The national nosocomial infection surveillance system identified \textit{Staphylococcus aureus} as the most common cause of hospital acquired infections occurring between 1990 and 1996 (Rubin et al., 1999). MRSA is one of the most bacterial infections in many hospitals and is the major causes of nosocomial pneumonia, surgical site and blood steam infections (Boyce, 1994). Antibiotic efficacy studies have elucidated that the prevalence of MRSA has increased steadily around the world, reaching 25-50\% of \textit{S. aureus} isolates in 1997. In some countries that have taken active preventive measures such as patient isolation to avoid MRSA spread, the incidence of MRSA can remain very low (Jones, 2001). The national nosocomial infection surveillance system, reported that approximately 60\% of all \textit{S. aureus} nosocomial infections in intensive care units were methicillin resistant in 2003, denoting an 11\% increase in resistance during preceding five years (NNIS, 2004). Compared with methicillin susceptible \textit{S. aureus} (MSSA) strains, infections caused by MRSA strains are associated with longer hospital stay, higher mortality, more days of antibiotic administration and higher costs. (Abramson and Sexton, 1999; Engemann et al., 2003; Farr, 2004; Kopp et al., 2004; Blot et al., 2002; Cosgrove et al., 2003).

There are two types of methicillin resistance in staphylococci: intrinsic high level resistance and intermediate resistance (borderline resistance, borderline susceptibility, diminished susceptibility). This classification is obtained by methods detecting resistance
patterns for example disk diffusion, kirby bauer; micro
dilution and E-test (Andrew and Simor, 2001). Intrinsice
high level resistance in MRSA is mediated by an abnormal
penicillin binding protein called PBP2A or PBP2’ encoded
by chromosomal mecA gene (Hackbarth and Chambers,
1989). Penicillin binding proteins are bound to cell
membrane and are the targets for all beta lactam
antibiotics and have an important role in bacterial cell
wall synthesis. PBP2A increases resistance to all beta
lactam antibiotics including Penicillins, Cephalosporins,
Cephamycins and Carbapenems by decreasing affinity for
binding these antibiotics. Also mecA contains plasmids
and transposons that make cross resistance to non beta
lactam antibiotics such as erythromycin, clindamycin,
garamycin, trimethoprim-sulfamethoxazole and
ciprofloxacin (Andrew and Simor, 2001). Some strains of
S. aureus produce large amounts of Penicillase that
hydrolyze the Penicillinase resistant Penicillins.
Susceptibility tests to oxacillin or Methicillin in these
strains may show reduction or borderline in susceptibility
and they are named as BORSA. The mechanism of
resistance of these mecA negative strains is production of
modified PBPs 1 and 2 with reduced affinities for beta
lactamase; production of a new beta lactamase; over
production of PBP4 or increased beta lactamase
production (Barber and Rozwadowska, 1948). The aim of
the study was to determine the frequency and type of
MRSA strains and antibiotic susceptibility in Al-Zahra
Hospital, Isfahan, Iran.

MATERIALS AND METHODS
This analytic descriptive survey was conducted from
2005 to early 2006 in Al-Zahra Hospital, a state runs
educational hospital in Isfahan, Iran. Patients who
contracted nosocomial infection after hospital admission
(absence of fever, signs and symptoms of infections
before hospitalization and at least 48 h after hospital
residence) were enrolled in the study. Specimens taken
from surgical wound, lower respiratory tract and blood
stream were cultured. A wound infection was identified
by the presence of purulent discharge from the incision
with erythematous cellulitis, induration or pain and
demonstrable fluid collection noted on ultrasound after
surgery. Aspirates were obtained by preparing the wound
area with alcohol, inserting a sterile needle through the
healing incision and aspirating fluid into a sterile syringe.
For the patients with nosocomial pneumonia (fever,
increase sputum production and infiltration in chest
radiography), specimens from lower respiratory tract were
obtained with Broncho Alveolar Lavage (BAL). For all of
the patients suspected with nosocomial infections
bloodstream cultured were performed too. All of the
specimens were cultured in blood agar media and
incubated at 35° for 18-24 h. After incubation, plates
were examined for the presence of moderately sized
smooth mauve colored colonies. All white colonies with
or without beta hemolysis were processed to rule cut
S. aureus. S. aureus was identified by gram stain, catalase,
slide coagulase test and growing in DNase, manitol salt
agar media. MIC of oxacillin on isolated bacteria was
determined by gradient concentration method (E-test; AB
BIODISK Co. Sweden). Quality control was tested by
staphylococcus ATCC29213. Minimum Inhibitory
Concentrations (MICs) were determined by Mueller
Hinton plates containing 2% NaCl which were inoculated
with a direct colony suspension equivalent to 0.5 Mac
Farland standards in accordance with the National
Committee for Clinical Laboratory Standards. The
breakpoints mentioned in the last edition of CLSI
tables M.A. were used to determine susceptibility and
resistance. The plates were incubated at 35°C for 24 h. All
strains with MIC of ≥4 μg for oxacillin were identified as
MRSA.

mecA and BORSA were detected by amoxicillin-
clavulanate strips (E-test; AB BIODISK Co. Sweden), a
beta lactam plus a beta lactamase inhibitor. Strains with
MIC of ≥4 μg for oxacillin and ≥8 μg for amoxicillin-
clavulanate were identified as mecA positive MRSA.
Other staphylococcus with MIC ≥4 μg for oxacillin and
≥4 for amoxicillin-clavulanate were identified as mecA
negative MRSA (BORSA).

Sensitivity of all isolates for vancomycin also was
tested by E-test (AB BIODISK Co. Sweden). Organisms
with MIC of ≥32 μg for vancomycin were known as VRSA
(Vancomycin Resistant Staphylococcus aureus).

The data were analyzed by the Statistical Package for
the Social Sciences (SPSS) version 13 and Who net
version 5. Comparisons were made by using Student's
t-test and comparisons of the categorical data by χ²
statistics or Fisher's test. A p-value < 0.05 was considered
to indicate statistical significance.

RESULTS AND DISCUSSION
One hundred and thirty four cultures of nosocomial
infections grew Staphylococcus aureus. 44 (32.8%) of
these cultures were positive for MSSA (MIC <4) and
90 (67.2%) for MRSA (MIC of oxacillin ≥4). Table 1
shows frequency of MSSA and MRSA in different
nosocomial infections. Although most of the MRSA
strains were obtained from surgical site infections but
there were no statistically significant differences between
types of Staphylococcus aureus growing from various
sites of infection. Sixty seven out of 90 (74.5%) MRSA
were mecA positive. Forty six out of 67 (68.7%) were from
surgical site infections, 6 (8.9%) from abscess aspiration, 11 (16.5%) from blood stream infections and 4 (5.9%) from bronchial lavage (Table 2). Twenty three (25.5%) of MRSA isolates were mecA negative BORSA. Seventeen (74%) of them were from surgical site infections, 3 (13%) from abscess aspiration and 3 (13%) from blood stream infections (Table 2). There was no statistically significant difference regarding isolation of mecA positive and mecA negative BORSA from various sites of infection. Only one (1.49) of the mecA positive MRSA had reduced susceptibility to vancomycin but all of the mecA negative MRSA (BORSA) were sensitive to it. Fong et al. (2000) reported 53.7% of staphylococcal isolates were designated as mecA positive that was higher than our study (50% of staphylococcal aureus and 74.5% of MRSA strains). In Levi et al. (2003) study 14 of 109 (12.8%) MRSA isolates were identified by conventional culture as borderline oxacillin-resistant S. aureus (BORSA), Gebhardt (2003) reported 15.8% frequency but in present study this frequency was 25.5% and was higher than their reports.

Vancomycin should be used for treatment of mecA positive staphylococcus infections in high risk patients. As vancomycin resistant strains are being encountered more often, great search is in progress for finding alternative therapies, for example the newly discovered drug, linezolid which is a member of a new class of antibiotics (Oxazolidinones) is being used (Murray et al., 1995). Daptomycin which is a newer semi synthetic glycopeptides antibiotic, could be used for the treatment of gram positive organisms and VRSA (Vancomycin Resistant Staphylococcus aureus) (Andrew and Simor, 2001). But infections with mecA-negative BORSA strains can be treated with Penicillinase-resistant Penicillins for example the Methicillin group or first and second generation cephalosporins (Barber and Rozwadowska, 1948). Unfortunately resistance of staphylococci to glycopeptides is rising. In the past few years, there have been reports from the United States, Japan and several European countries indicating reduced susceptibility (intermediate resistance) of S. aureus strains to vancomycin and other glycopeptides (Smith et al., 1999). Clinical infections with these strains lead to significant morbidity and prolonged antimicrobial therapy. When vancomycin is used for a long time, bacterial cell wall proteins may be modified which is probably responsible for the emergence of Glycopeptide resistance in these isolates (Andrew and Simor, 2001). We found only one of MRSA specimens was resistant to vancomycin. Vancomycin use increases as MRSA infections become more prevalent in our hospital and all around the worlds. This results in increased selective pressure for the emergence of Vancomycin resistant organisms such as Vancomycin-resistant Enterococcus and Vancomycin-resistant S. aureus.
Because one forth of our staphylococcus strains are meca negative BORSA and there is no alternative for vancomycin in treatment of meca positive MRSA and Enterococcus spp. in our hospital, Vancomycin should be reserved only for life threatening infections due to meca positive MRSA. So we recommend the use of vancomycin as empiric antibiotic therapy in suspected Staphylococcus aureus nosocomial infections until susceptibility results became available.

REFERENCES


